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In re application of:

QI *et al.*

Serial No. 10/804,762

Filing Date: March 19, 2004

For: *Specific Inhibition of Allorejection*

Examiner: KELLY, Robert M.

Art Unit: 1633 Confirmation No. 8100

CERTIFICATE OF ELECTRONIC TRANSMISSION

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Dated: May 25,2007

Signed: Ann-Ellice Farlee

DECLARATION
Pursuant to § 1.132

The undersigned, Dr. Uwe Staerz, hereby declares as follows:

1. I received my Ph.D. in Immunology in 1986. A copy of my curriculum vitae is attached. I am employed by Isogenis, Inc., the assignee of the above-referenced application and currently serve Chief Scientific Officer of the company.

2. I have read and am familiar with the disclosure in the above-referenced application and have reviewed as well the Examiner's comments in his most recent office action mailed November 30, 2006.

3. I understand that the Examiner has a number of concerns regarding the extent to which the claimed invention works. Specifically, the Examiner is concerned about whether immune responses other than adaptive CTL immunity are affected; about the timing of the immune inhibitory effects; and again about the level of CD8 alpha chain expression obtainable in allograft tissues. I also understand that the Examiner is concerned about the novelty of using a vector encoding CD8 alpha alone instead of a vector encoding CD8 alpha and beta chains *in vivo*. I will address the latter concern first by beginning with a brief overview of the state of the art in CD8 veto technology as of our priority date (March 19, 2003).

4. By 2003, veto was understood to be a specific immune inhibitory function exerted by hematopoietic cells, such as cytotoxic T lymphocytes and certain bone marrow-derived cells. Indeed, the scientific community in 2003 considered that only hematopietic cells could exert a

veto effect. All approaches under investigation at the time taught the use of T lymphocytes or bone marrow derived dendritic cells. See, e.g., Asiedu, C., et al., Immunologic Research, 2002, 26/1-3: 297-302, providing in its introductory paragraph "The tolerogenic activity of donor BMCs is credited to the veto effect, which is the functional inactivation of donor derived CTL precursors (CTLp) by an alloantigen-bearing cells that is recognized by CTLp. The alloantigen-bearing cell is referred to as a veto cell." See also, e.g., Bachar-Lustig, E., et al. Blood, 2003, 102: 1943-1950 (Abstract Lines: 1-5): "Several bone marrow cells and lymphocyte subpopulations, known as 'veto cells', were shown to induce transplantation tolerance across major histocompatibility antigens."

5. While the underlying mechanism of veto was not known in 2003, the scientific community did not believe the CD8 alpha chain could work as a veto effector as it is surface bound. A long-standing theory championed a direct signaling function of the CD8 molecule (see, e.g., Sambhara, S.R., R.G. Miller, Science, 1991, 252: 1424-27). The Sambhara study used a CD8 alpha chain gene to transfect a T cell line *in vitro*, which resulted in the production of both soluble and surface-bound forms of CD8 molecules (see, e.g., Tomkinson, B.E., et al. J. Immunol. 1989, 142: 2230-2236). An inhibitory effect was observed for which soluble rather than surface-bound CD8 molecules appeared to be responsible. That is, it was shown that another soluble ligand with binding characteristics of CD8, a soluble anti-MHC class I monoclonal antibody, was able to delete T cells. Furthermore, others independently showed that soluble preparations of monoclonal antibodies binding the MHC class I α3-domain eliminated lines of T cells (see. e,g., Woodle, S.E., et al., J. Immunol., 1997, 158: 2156-2164). The inevitable conclusion from these studies was that soluble forms of CD8 were needed to effect veto.

6. Later mechanistic studies suggested that veto immune inhibition was mediated by the expression of the Fas ligand (FasL) on the surface of the veto cells or by the secretion of TGF-β1. FasL as the veto effector was championed by Drs. Reich-Zeliger (see, e.g., Reich-Zeliger, S., et al., Immunity, 2000, 13: 507-515) and Rich (see, e.g., Rich, R.F., W.R. Green, J. Immunol., 2002, 168: 2751-2757); while TGF-β1 was favored by Dr. Asiedu (see, e.g., Asiedu, C., et al., Immunologic Research, 2002, 26/1-3: 297-302). This proposed mechanism relied not only on the effector being soluble, but also on the veto cells being hematopoietic cells, capable of producing FasL or TGF-β1 after engaging the CD8 α:β heterodimeric molecule.

7. The disclosure in U.S. Patent Application No. 2002/0127205, Edge et al. (hereinafter "Edge") is cursory at best and does not in my opinion undercut the conventional view that soluble CD8 is preferred. Edge also teaches that one should employ a CD8 α:β heterodimer as opposed to the CD8 alpha chain alone. In particular, Example 4 of Edge instructs

that the CD8 construct is prepared using the human CD8 gene based on the nucleotide sequences provided in Shuie (1988) J. Exp.Med. 168: 1993-2005 and Nakayama (1980) Immune Genetics 30: 303-307, which provide the human CD8 genes for the alpha and beta chains, respectively. Moreover, using the genomic CD8 alpha sequence taught by Edge in a construct would result in both soluble and cell-surface expressed versions of the CD8 alpha chain, due to the presence of introns and exons in the genomic sequence leading to alternative splicing.

8. Turning next to the Examiner's concern regarding the extent of the immune inhibitory effects, the experimental data will now be further explained. The Examiner queries whether antigen specific CTLs can be inhibited based on data in the subject application based on the data in Figure 5A. It needs to be noted that in this initial experiment the cell population employed was a relatively undefined mixed lymphocyte culture which included effector cells such as NK cells and lymphokine-activated killer cells in addition to the T cells noted on the graph, and it is likely these other cell types that were responsible for the efficient lysis observed.

9. Based on our results we surmised that the lack of definition of the effector cell population was a problem and therefore a second experiment was designed that limited the population solely to activated cytotoxic T cells. The redesigned protocol employed an antigen-specific T cell clone and, as shown in Figure 5B, the killing of peptide-coated (i.e. antigen-coated) targets was inhibited significantly. Thus, I believe the data collectively establishes that fully-activated antigen-specific T cells are inhibited as well as allogeneic T cells. Moreover, I would also point out the data and experiment represented in Figure 9 of the patent specification, which further establishes that CD4+ T cells can also be inhibited by the subject methods. This was a surprising result in that the soluble CD8 approaches taught in the prior art were described as ineffective in inhibiting this cell population.

10. The Examiner also queries whether intravascular vector injection at a site proximate to the target cells would work after 2 days post-transplant, basing this statement on the *in vitro* studies in Sambhara (Sambhara, S.R., R.G. Miller, Science, 1991, 252: 1424-27). *In vivo* rejection, however, shows different timing. For instance, in the case of allogeneic kidney rejection, few reactive T cells are seen at day 2 post-transplant whereas at day 5 massive infiltration can be found (see, e.g., Manca, F., et al., Scand J Immunol., 1987, 25: 255-64). Accordingly, a day 2 cut-off is not warranted for *in vivo* applications. Furthermore, data presented in Figure 5B of the subject application suggest that fully activated T cells can be inhibited by CD8-expressing cells, as discussed above. Accordingly, delivery of a veto vector will very likely be effective beyond 2 days into the T cell effector/rejection phase, when full activation occurs.

11. Finally, I again address the Examiner's concern about the level of CD8 alpha chain expression in an allograft. I understand the Examiner is concerned that certain tissues are refractory to transformation (based on Example 3 of the subject specification). The Examiner further postulates that either clearing or a quick pass-through would prevent adequate transduction. To the contrary, the Example 3 addresses both of these concerns, as explained in detail below.

12. Example 3 of the subject specification describes *in vivo* administration of adenoviral vectors carrying CD8 alpha chains for inhibiting rejection of skin grafts (paragraphs 0216 to 0217 of the published application). Properly interpreted, these experiments demonstrate that *in vivo* administration of the veto-carrying vectors was effective in reducing and delaying rejection of skin grafts, despite low transduction frequencies.

13. Adenoviral vectors injected into the skin of donor animals were preferentially taken up by skin cells around hair follicles but gave an overall transduction frequency of less than 0.5%. Full-thickness skins were transduced either with a vector carrying the mouse CD8 alpha chain (mAdCD8) or a control carrying β-galactosidase (AdLacZ) and then transplanted onto allogeneic hosts. In spite of the low frequency of transduced donor cells, a significant delay in rejection was seen. The specification at paragraph 0127 provides that "...tolerance was not induced and the skins eventually succumbed to rejection, apparently due to the later-determined fact that the majority of skin cells are refractory to infection with adenoviral vectors." This language, while correct in a literal description of the experiment, may not have fully conveyed the true effectiveness of the veto phenomenon that occurred even with low transduction frequency. I refer you to Figures 1 and 2 below, which evidence our actual results and unmistakably show delayed and reduced rejection due to the veto effect. Also, these results further support the data discussed in my previous Declaration of September 29, 2006.

14. Furthermore, other infusion strategies known in the art can deliver vectors to tissues *in vivo* in a highly localized fashion. In the case of kidney delivery, for example, both intra-arterial delivery or delivery in a retrograde fashion through the ureter have been successfully employed. See, e.g., Imai, E., Y. Isaka, Kidney Int., 1998, 53: 264-272 and Chen, S., et al., J. Am. Soc. Nephrol., 2003, 14: 947-958. Indeed, *ex vivo* transfusion protocols have been developed using this "kidney loading" approach to optimize transduction. For example, pig kidneys transduced with mAdLacZ using a 120 min loading regimen with a clamped kidney vein, at 4°C, expressed high levels of the transgene β-galactosidase. Results are reproduced below as Figure 3. Based on these results, it is evident that arterial delivery does not have to proceed as a "quick pass through the organ." Finally, another workable approach that can be envisioned involves extracting and recycling the vector infusate after kidney pass-through by

placing a catheter in the kidney vein. These techniques promise increased transduction and therefore even better tolerance using the veto-carrying vectors of our application.

Figure 1 Transplantation of AdLacZ (A) or mAdCD8 (B) transduced full-thickness Balb/c skin onto C56Bl/6 recipients. The pictures were taken day 12 after transplantaton.

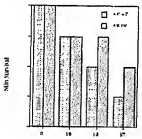

Figure 2 Survival of transduced (as indicated) full-thickness Balb/c skin on fully allogeneic C57Bl/6 recipients.

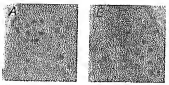

Figure 3: Mock-infected (A) or AdLacZ-transduced (B) kidneys tested for the expression of beta-galactosidase.

14. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dr. Uwe Staerz

05-25-07
Date